Clinical Study Report

A phase I/IIa, open-label, escalating dose, pilot study to assess the effect, safety, tolerability and pharmacokinetics of multiple subcutaneous and intravenous doses of PRO044 in patients with Duchenne muscular dystrophy

CONFIDENTIAL

Study no.	PRO044-CLIN-01
EudraCT Number	2009-013762-63
Investigational product	PRO044
Indication	Duchenne muscular dystrophy
Development phase	I/IIa
First patient included	10 March 2010
Last patient completed	28 May 2013
Date of report	28 May 2014
Date of earlier reports	Not applicable

Sponsor

Prosensa Therapeutics B.V.

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This study was performed in compliance with Good Clinical Practices (GCP), including the archiving of essential documents.

2 Synopsis

<table>
<tr><td>Name of Sponsor/Company
Prosensa Therapeutics B.V.
J.H.Oortweg 21
2333 CH Leiden
The Netherlands</td><td>Individual study table
Referring to part of the dossier</td><td>(For national authority use only)</td></tr>
<tr><td>Name of finished product
PRO044</td><td>Volume</td><td></td></tr>
<tr><td>Name of active ingredient
h44AON188</td><td>Page</td><td></td></tr>
<tr><td colspan="3">Title of study
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<tr><td colspan="3">Investigators
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J.J.G.M. Verschuuren, M.D. Ph.D., Leiden University Medical Center, Leiden, the Netherlands.
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<tr><td colspan="3">Study center(s)
S.Anna Hospital, Ferrara, Italy.
The Queen Silvia Children’s Hospital, Göteborg, Sweden.
Leiden University Medical Center, Leiden, the Netherlands.
UZ Leuven, Leuven, Belgium.</td></tr>
<tr><td colspan="3">Publication (reference)
Not applicable.</td></tr>
<tr><td colspan="2">Studied period
First patient enrolled: 10 March 2010
Last patient completed: 28 May 2013</td><td>Phase of development
I/IIa</td></tr>
<tr><td colspan="3">Objectives
• To preliminarily assess the effect of PRO044 at different dose levels in subjects with Duchenne muscular dystrophy.
• To assess the safety and tolerability of PRO044 at different dose levels in subjects with Duchenne muscular dystrophy.
• To determine the pharmacokinetics of PRO044 at different dose levels after subcutaneous and intravenous administration in subjects with Duchenne muscular dystrophy.</td></tr>
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Methodology

A phase I/IIa, open-label, escalating dose, multiple dose, pilot study.

Subjects received 1 subcutaneous (SC) injection or intravenous (IV) infusion of PRO044 per week for a period of 5 weeks (i.e., 5 injections/infusions in total). Each consecutive group consisted of 3 subjects who were treated with 0.5 mg/kg SC (Cohort 1), 1.5 mg/kg SC (Cohort 2), 5 mg/kg SC (Cohort 3), 8 mg/kg SC (Cohort 4), 10 mg/kg SC (Cohort 5), 12 mg/kg SC (Cohort 6), 1.5 mg/kg IV (Cohort 7), 5 mg/kg IV (Cohort 8), and 12 mg/kg IV (Cohort 9) respectively. The subjects treated in the last 3 cohorts, Cohorts 7, 8 and 9 also received PRO044 treatment in one of the previous SC cohorts.

Effect and safety assessments were done at regular intervals. Prior to each dose escalation, the safety data of at least 3 administrations of PRO044 per subject were reviewed. If potential dose-limiting toxicity occurred in 1 subject within a dose group, the group was to be expanded to 6 subjects. If similar dose-limiting toxicity occurred in 2 or more subjects, the inclusion of new subjects was to be discontinued at that dose level. Furthermore, the inclusion of new subjects into the study could be stopped, or the study could continue with a lower intermediate dose level, or by expanding the cohort of the previous dose level to 6 subjects. The dose level of the group with a dose level one step below the group with similar dose-limiting toxicity in 2 or more subjects was to be considered the maximum tolerated dose.

Number of subjects (planned and analyzed)

18 subjects were planned; 18 subjects were treated, completed the study and were analyzed.

Diagnosis and main criteria for inclusion

1. Boys aged at least 5 and not older than 16 years on the day of first drug administration.
2. Duchenne muscular dystrophy resulting from a mutation correctable by treatment with PRO044.
3. Life expectancy at least 6 months after inclusion in the study.
4. No previous treatment with investigational medicinal treatment within 6 months prior to the start of the (pre)-screening for the study.
5. No previous treatment with idebenone within 6 months prior to the start of the (pre)-screening for the study.
6. Glucocorticosteroid use which was stable for at least 2 months prior to first drug administration.

Test product, dose and mode of administration, batch number

The study drug for Cohorts 1–5, batch 07P44-001, 2'-*O*-methyl-phosphorothioate oligonucleotide PRO044 was manufactured by Synco BioPartners B.V., the Netherlands, and prepared as a solution for injection (92 mg/mL). The study drug was delivered as solution for injection in vials containing 0.5 mL with 46 mg PRO044.

The study drug for Cohorts 6–9, batch 10P44-004, 2'-*O*-methyl-phosphorothioate oligonucleotide PRO044 was manufactured by GSK Parma, Italy, and prepared as a solution for injection (200 mg/mL). The study drug was delivered as solution for injection in vials containing 1.0 mL with 200 mg PRO044.

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Duration of treatment

Maximum of 28 weeks for the SC cohorts, consisting of approximately 10 weeks (pre)-screening, 5 weeks of treatment plus 13 weeks follow-up.

Maximum of 22 weeks for the IV cohorts, consisting of approximately 4 weeks screening, 5 weeks of treatment plus 13 weeks follow-up.

Provided the subject and/or his parents, the Investigator and the Sponsor all agreed that drug administration appeared to improve the clinical status of the subject without direct safety or tolerability concerns, subjects may be offered the option to continue treatment after the 13-week follow-up period in a future study.

Reference therapy, dose and mode of administration, batch number

Not applicable

Criteria for evaluation

Effect parameters

- Presence of (Becker Muscular Dystrophy like) dystrophin expression after treatment (in muscle biopsy).
- Muscle function (timed tests and 6-minutes walk test).
- Muscle strength (handheld myometry and spirometry).

Safety

- Adverse events.
- Local tolerability.
- Vital signs.
- Physical examination.
- Safety biochemistry and hematology parameters.
- Coagulation (activated partial thromboplastin time [aPTT], Pro thrombin time [international normalized ratio, INR], and fibrinogen).
- Cystatin C.
- Urinalysis (microscopy on sediment, quantitative albumin, protein, glucose, creatinine, alpha$_1$-microglobulin, and Kidney Injury Molecule [KIM-1]).
- Complement component 3 (C3) and Complement split products (C3a/SC5b-9/Bb).
- Cytokines (interleukin-6 [IL-6], tumor necrosis factor-α [TNF-α]) and chemokine (monocyte chemotactic protein [MCP-1]).
- Antibodies to dystrophin.
- ECG parameters.
- Concomitant medication.

Pharmacokinetics

- PRO044 plasma profile characterized by: time to achieve maximum plasma concentration (t_{max}), maximum plasma concentration (C_{max}), area under the plasma concentration-time curve (AUC), plasma clearance (CL or CL/F) and terminal half-life (t½) by non-compartmental analysis. Three compartmental modelling was used in some individuals receiving IV infusion because the sampling at the end-of-infusion was missed or deemed unreliable.
- PRO044 levels in muscle tissue.
- Urinary excretion of PRO044 and metabolites.

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Statistical methods

Sample size justification

The sample size has been chosen based on practical grounds rather than on statistical grounds, because of the low prevalence of Duchenne muscular dystrophy (DMD) subjects carrying a mutation correctable by PRO044.

Statistical analysis

All effect and safety data were listed and summarized per dose group. No formal statistical analyses were conducted. Data was scrutinized for effect and signs of potential safety or tolerability issues.

Plasma concentration versus time profiles of PRO044 was analyzed to calculate the pharmacokinetic parameters. For some IV subjects non-compartmental analysis was not possible due to sampling issues; for these the pharmacokinetic parameters were estimated using a three-compartmental model.

Summary – conclusions

Effect data

Caution should be applied to interpretation of these data given the small number of subjects in each cohort, the variability of the data and the lack of placebo control.

In general, naturally occurring (treatment-unrelated) exon skipping in the muscle biopsies of these subjects appears to be higher than in subjects that have deletions that may be correctable by exon 51 skipping as seen in the trace dystrophin expression and number of revertant fibers. Therefore the muscle biopsies obtained could only be analyzed using immunofluorescence. Not all muscle biopsies could be analyzed. For subjects with evaluable results, an increase from Baseline in dystrophin intensity was detected in 46% (6/13) subjects in the SC dose groups and in 75% (6/8) subjects in the IV dose groups. The increase in dystrophin did not appear to be dose-related, but was more subject dependent. PRO044 concentrations determined in the muscle tissue were variable between subjects, but there appeared to be a general trend towards a small increase in PRO044 concentration with increased dose. Overall, the PRO044 muscle concentrations detected after IV treatment were similar or slightly higher than after SC treatment.

No clear change between dose groups or within dose groups was observed following SC or IV PRO044 administrations for the muscle function tests: 6 minutes walking distance test, 10-meter walk/run, timed rising from floor and stair climb during 5 weeks of treatment.

Over the short duration of the trial (5 weeks treatment), the efficacy parameters (timed function tests [rise from floor, 10 meter walk/run, 4-stair climb], 6-minute walk test, handheld myometry, and spirometry) showed variable changes with no clear dose response between the different dose groups.

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Safety data

SC administration of PRO044 dosed at 0.5, 1.5, 5, 8, 10 and 12 mg/kg and IV administration of PRO044 dosed at 1.5, 5 and 12 mg/kg for 5 weeks appeared to be generally well tolerated, and no subjects withdrew from the study prematurely due to treatment-emergent adverse events (TEAEs).

The most common TEAEs in the SC dose groups were related to administration site events with injection site hematoma and erythema being reported by 89% (16/18) subjects and 78% (14/18) subjects, respectively. The most common TEAEs in the IV dose groups were related to injury, poisoning and procedural complications, with contusion being reported by 56% (5/9) subjects. There were no severe TEAEs in either the SC or IV dose groups, and all treatment-related TEAEs were classified as mild intensity, except for a proteinuria event in Subject 0207 in the 12 mg/kg (Cohort 9) IV dose group, which was moderate in intensity and considered to be possibly related to study drug.

Two subjects had a total of 4 serious AEs, none of which were considered by the Investigators to be treatment-related.

Based on review of the AE and laboratory data, there is no evidence of overt drug-induced hepatotoxicity. Subjects in both the SC and IV PRO044 dose groups had elevated alanine aminotransferase, aspartate aminotransferase, creatine kinase and lactate dehydrogenase levels at Baseline and at all time-points post-Baseline, which are not unexpected in DMD subjects, as these enzymes “leak” from the affected muscle. No meaningful changes in bilirubin or changes in gamma glutamyl transferase or glutamate dehydrogenase levels were observed, except for 1 subject in the 5 mg/kg (Cohort 8) IV dose group who had clinically significant glutamate dehydrogenase levels at Weeks 4 and 9.

Within the SC dose groups, there was evidence of mild proteinuria both prior to and post-dose, and an isolated moderate event of orthostatic proteinuria was reported. Within the IV dose groups, only 1 moderate proteinuria event was reported. No changes in α-1 microglobulin values were observed.

There were no apparent clinically significant effects on coagulation (as determined by aPTT, INR and fibrinogen).

There was evidence of minor reductions in complement factor C3 values in both the SC and IV PRO044 dose groups. Complement splits products (SC5b-9 and Bb) remained generally unchanged over time and non-clinically significant C3a levels below the lower limit of normal were observed both prior to and post-dose.

There was no evidence of clinically relevant effects on the inflammatory and immune response (as determined by IL-6, and TNF-α). Investigator defined, clinically significant elevations in MCP-1 were observed following IV PRO044 administration, but rapidly reversed once treatment was stopped.

Thrombocyte counts and serum cystatin C values were mostly within the normal range, and no clinically relevant changes were observed.

A total of 4 subjects from the PRO044 SC dose groups and 5 subjects from the PRO044 IV dose groups met a laboratory safety parameter stopping and follow-up criterion, study drug administration was not stopped for any of these subjects because the last planned PRO044 dose had already been given prior to meeting the criteria.

Local injection/infusion site reactions including: erythema, edema, pain, induration and bruising reactions were more frequently reported in the PRO044 SC dosing groups compared to the PRO044 IV dosing groups.

No subjects had any detectable antibodies to dystrophin in blood.

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Pharmacokinetic data

After single and multiple SC injections of 0.5, 1.5, 5, 8, 10 and 12 mg/kg, or 3-hour IV infusions with 1.5, 5 and 12 mg/kg, PRO044 was quickly absorbed with peak levels mostly reached between 1-2 hours after SC dosing. Thereafter, PRO044 was rapidly distributed and cleared with a decline in plasma levels to less than 3.4% of the C_{max} at 24 hours and to less than 0.3% of the C_{max} at the end of the dose interval (i.e. trough levels at 7 days after injection) for SC administration. Plasma levels after a 3-hour IV infusion decreased even more rapidly to less than 0.5% of the C_{max} at 24 hours and to less than 0.2% of the C_{max} at the end of the dose interval. Consequent to the low residual plasma levels, peak plasma levels remained similar upon 5 repeated administrations, and total plasma exposure as determined by $AUC_{(0-24h)}$ and $AUC_{(0-7d)}$ remained similar as well. However, the trough plasma levels increased upon repeated dosing over 5 weeks.

C_{max} and AUC parameters increased with dose over the administered range for SC and IV administration routes. For SC administration, the increase appeared to be more than dose proportional from 0.5 mg/kg to 1.5 mg/kg and slightly less than dose-proportional between 1.5 and 5 mg/kg and between 8 and 10 mg/kg. For IV dosing the increase was also less than dose proportional between 1.5 and 5mg/kg, but a proportional change was observed between 5 and 12 mg/kg. Taking into account that the number of subjects per dose group was limited and that the total absolute dose depended on the weight of subjects which differed considerably for the different dose groups, overall the data suggests dose linearity for plasma exposure.

Plasma bioavailability was complete for SC administration. The plasma clearance was high and variable. For IV administration the geometric means for CL/F, estimated from the first administration, ranged between 2310 and 7571 mL/h at 12 and 5 mg/kg, respectively. For SC administration, the CL/F was in a similar range with geometric means between 3375 and 7481 mL/h at dose levels of 1.5 and 12 mg/kg, respectively.

PRO044 was generally still detectable in plasma at 13 weeks after the last dosing. These plasma levels were low and variable. The t½ could only be estimated for a limited number of subjects (50% [9/18] subjects in the SC dose groups and 44% [4/9] subjects in the IV dose groups). For subjects with available data, the geometric mean was 1470 hours (61 days) and ranged from 916 to 1925 hours (38 – 80 days).

In urine, around 55% of drug was excreted as 3' n-x metabolites (up to n-3) from which n-1 was most prominent. The percentage of total dose excreted in urine over 24 hours after administration of PRO044 appeared to vary from 3% to 29% of the total dose.

Conclusion

PRO044 administered SC at dose levels of 0.5, 1.5, 5, 8, 10 and 12 mg/kg was generally well tolerated. PRO044 administered IV at dose levels of 1.5, 5 and 12 mg/kg was also well tolerated.

These results support future long-term studies with this compound to explore its therapeutic potential in a subset of DMD patient's with appropriate mutation(s).

DATE OF THE REPORT

28 May 2014